Use of PCR for the clinical diagnosis of Legionnaires' disease

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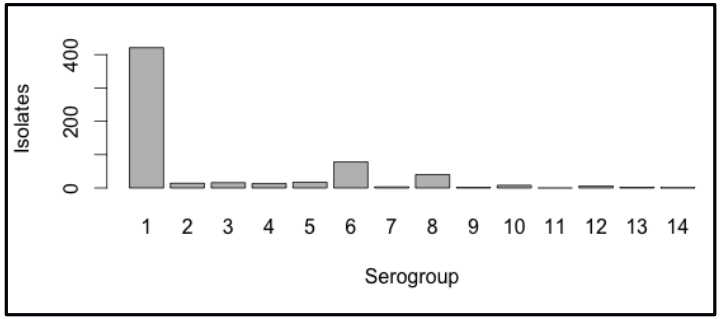
Outline

- Background
- Specimens and intended uses
- PCR
  - Workflow
  - Targets
- Performance evaluation
- Test availability

Background

- *Legionella*
  - >60 species known
  - More than a third of these species connected to human disease
  - *L. pneumophila*
    - 17 serogroups
    - Lp1 (serogroup 1) - >80% cases

No. clinical isolates isolated (2000-2015)

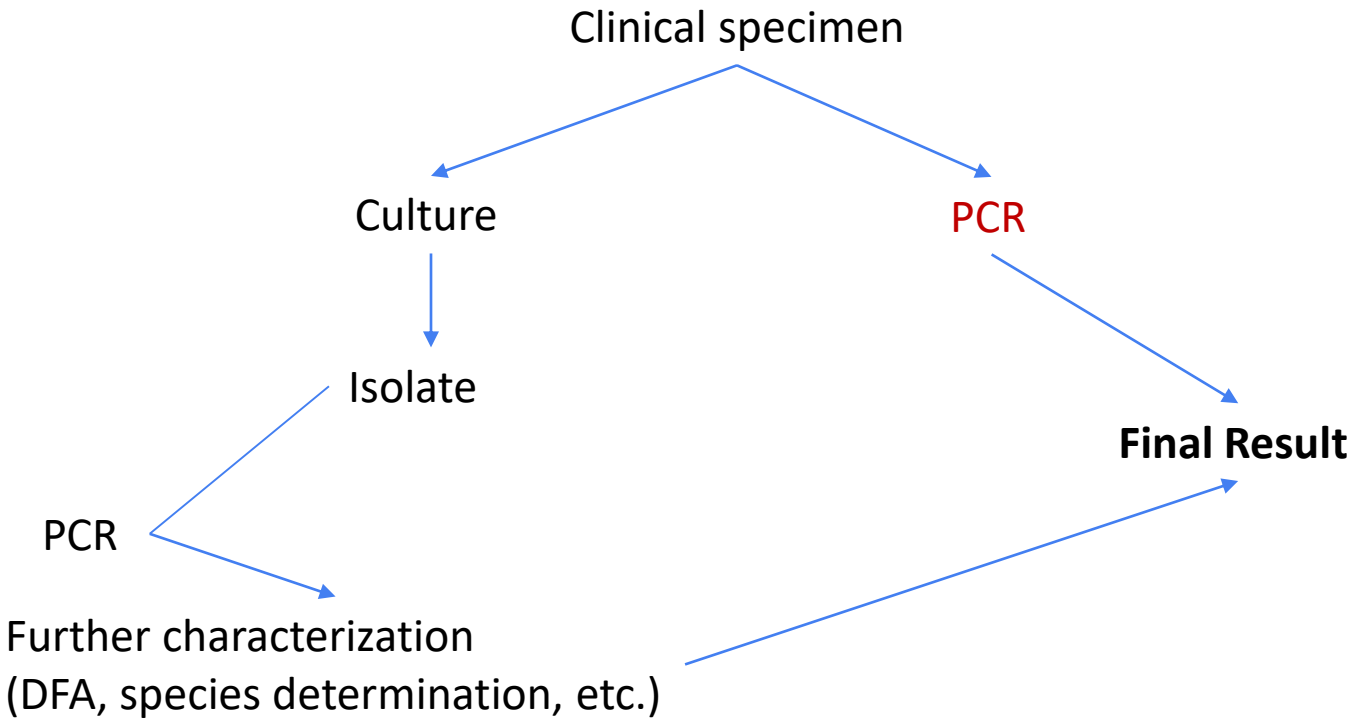


Specimens

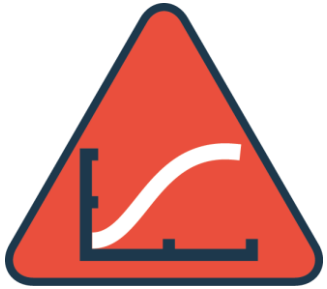
- Sputum
  - Store frozen!
  - Do not reject “low quality” specimens
- Bronchoalveolar lavage, aspirates, pleural fluid, etc.
  - Concentrate specimen
- Lung tissue
  - Homogenize
- NP/OP swabs are not recommended
  - Poor sensitivity

- Lower respiratory tract specimens
- Clinical specimens are precious in LD investigations – only means to obtain an isolate

Role of PCR in CDC lab



PCR – Workflow



Process specimen

- DTT digest (sputum)

Extract DNA

- Automated
- Manual

Amplify

- PCR set-up
- Instrumentation

Analyze data

- Set threshold
- Generate report

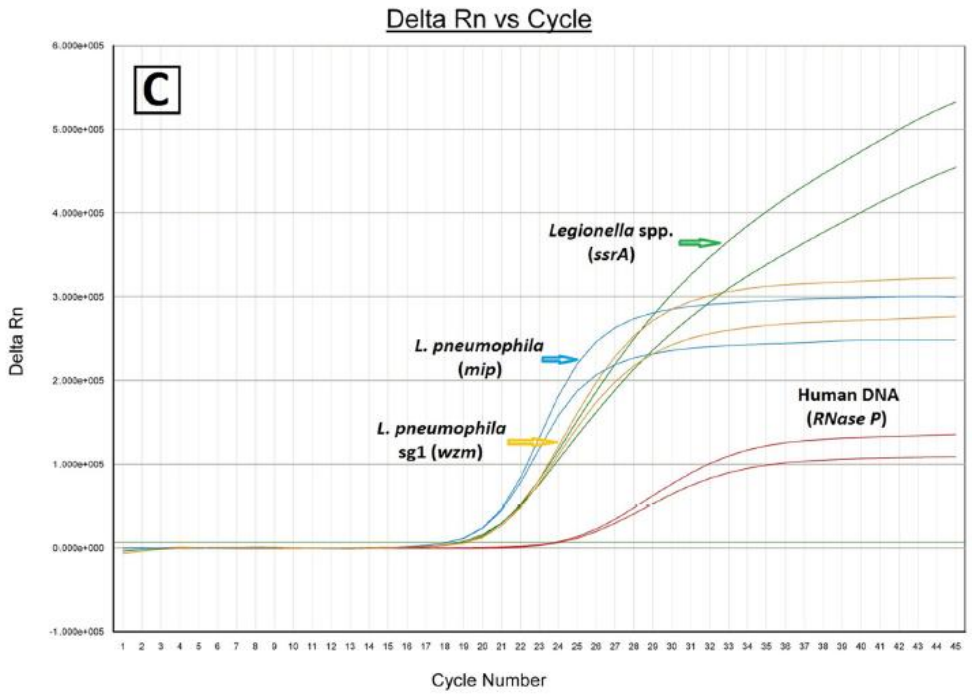
A note…

PCR on clinical specimens is very different compared to PCR on environmental samples:

1. Environmental samples are far more likely to contain *Legionella* DNA.
2. Environmental samples could contain PCR inhibitors that affect reaction. Inhibition controls for every samples is a must.

PCR Targets

- *Legionella* spp.
  - *ssrA* – tmRNA
- *L. pneumophila*
  - *mip*
- Lp1
  - *wzm*
- Extraction control
  - Rnase P (human)



Benitez and Winchell. 2013. J. Clin. Microbiol. 51:348-351.

Performance evaluation – experience in CDC lab

- Review of 96 clinical specimens submitted 2016-2018
  - 77% of specimens were sputa
- PCR positives = 36
  - Leg spp = 3
  - Lp = 4
  - Lp1 = 29

PCR (any target)	Culture: Positive	Culture: Negative
Positive	17	19
Negative	0	60

Performance evaluation – selected published reports

- Diederen et al (2008) – 2 assays detecting either 16S or *mip*
  - LD diagnosed in more cases by combination of PCR and UAT
- Chen et al (2015) – using Lp PCR test
  - 32 cases with proven or suspect LD – 92% sensitivity by PCR
  - No false-positive PCR result observed
- Avni et al (2016) – systematic review of 28 studies
  - PCR resulted in additional diagnosis of 18-30% of LD cases
  - Sensitivity of PCR on blood and urine samples very low (<50%)
- Peci et al (2016) – used Leg spp and Lp PCR at single PHL
  - Sensitivity of UAT vs PCR: 74.7%
  - Sensitivity of culture vs PCR: 45.2%

Availability of PCR assays for clinical use

- No commercially-available FDA-cleared PCR assays for *in vitro* diagnostic use
- Laboratory-developed tests
  - Need internal validation to use for clinical specimens in compliance with CLIA regulations
  - Test directories include PCR at various large reference labs:
    - http://ltd.aruplab.com/Tests/Pub/2010125
    - http://www.questdiagnostics.com/testcenter/BUOrderInfo.action?tc=15062&labCode=AMD
    - https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/89564
  - Some availability in hospital laboratories
  - Public health laboratories

Summary

- A validated PCR is an important diagnostic assay for LD
- Increased sensitivity compared to culture
  - Esp. if antibiotic treatment has begun, long-term suboptimal storage, etc.
  - High negative-predictive value
- May capture cases not detected by UAT alone
  - Due to non-Lp1 strains
  - Ideally, UAT and lower respiratory tract specimen should be tested (PCR and/or culture)
- Instrumentation and assays widely available
  - Significantly less technical expertise required compared to culture

QUESTIONS?

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.